
Regulation of Calcium and Phosphate Metabolism

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Abstract

This is an up-to-date discussion of calcium and phosphorus metabolism and the many regulators both old and new involved in the control of these minerals. The author provides a discussion of dietary sources of these minerals, their intestinal and renal handling. He focuses on the well-established regulators such as parathyroid hormone and vitamin D and also provides new insights into this regulation by the new compounds FGF-23 and Klotho. The author also highlights the molecular sensors for calcium and phosphorus, membrane receptors, and their importance in controlling mineral metabolism at the intestine, kidney, and bone. He ends the chapter with discussions of how macronutrients such as carbohydrates and proteins influence the overall regulation of these minerals.

Keywords

Metabolism • Regulation • Hydroxyapatite • Calcium • Phosphorus • Parathyroid hormone • PTHrP • CYP27B1 gene • Calbindin • CYP24A1 gene • Calcitriol • 1,25(OH)₂ vitamin D₃ • 24,25(OH)₂ vitamin D₃ • FGF-23 • Klotho • Calcium-sensing receptor • Synthesis • Secretion • GALNT3 gene • TRPV5,6 channels • Depolarization • Intestinal transport • Ionized calcium • Phosphoric acid • Phosphate carrier • Renal handling calcium and phosphorus • Intracellular calcium function • Extracellular calcium function • Bone mineral • Hypocalcemia • Hypercalcemia • Secretion • Osteoblasts • Osteoclasts • Kidney reabsorption • Intestinal absorption • 25-Hydroxyvitamin D₃ • 1 α -Hydroxylase • IGF-1 • Vitamin D binding protein • Osteomalacia • Phosphatonin • Mutations calcium-sensing receptor • Phosphate-sensing mechanisms • Calcium transport mechanisms • Intestinal calcium transport • Macronutrients • Intestinal phosphate absorption • Renal control mechanism of calcium and phosphate transport

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- Hormonal regulation of calcium renal transport • Calcitonin • Renal tubular phosphate transport • Renal proximal tubule • Cartilage and bone and mineral regulation • Bone resorption • Remodeling • Modulators bone resorption • Calcitonin • Estrogen • Leptin • β -adrenergic signaling • Bone formation • Mineralization • SIBLING • Rickets • Hypophosphatemic rickets • ADHR • XLH • ARHR • Bone calcium-sensing receptor • Thick ascending limb • Phosphate transporters (Npt2a and Npt2c and PiT-2) • Secreted frizzled-related protein-4 • Na/H exchanger regulatory factors • Null mice • Apoptosis • Cathepsin proteases • Sclerostin • Hypocalciuric hypercalcemia • Cytokine TNF- α • RANK ligand • Tissue nonspecific alkaline phosphatase • Dentin matrix protein-1 • Matrix extracellular phosphoglycoprotein • PHEX • Phosphatonin • Ossification

Introduction

The metabolisms of calcium and inorganic phosphate are intimately connected as, respectively, the cationic and anionic components of crystalline hydroxyapatite, which confers rigidity on the bone matrix (Fig. 2.1). In this chapter, the author considers the biological processes that underlie the balances of Ca^{2+} and phosphate as well as the key points of exchange in the gut, kidney, and bone, together with their regulation. These balances depend on dietary composition and the

effectiveness with which calcium and phosphate are released from ingested food. They also depend on the efficiencies of intestinal absorption, glomerular filtration, and renal tubular reabsorption along with rates of transfer between the blood and the bone mineral store as well as the magnitudes of unavoidable losses associated, for example, with small and large intestinal secretions. Regulation of calcium and phosphate metabolism depends on several important hormones, chiefly parathyroid hormone (PTH), $1,25(\text{OH})_2$ vitamin D_3 (calcitriol), and fibroblast growth factor-23

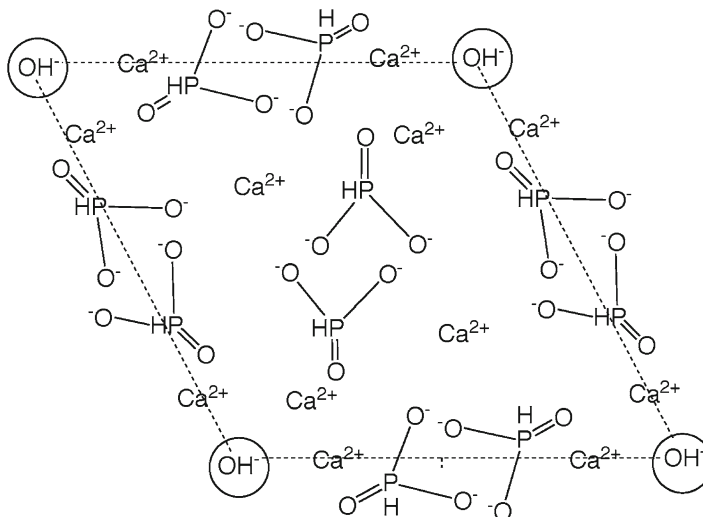


Fig. 2.1 Partial structure of the bone mineral hydroxyapatite. A view of the repeating structural unit of hydroxyapatite based on the analysis in [269]. The corners of the

repeating unit are occupied by hydroxyl ions. The hydroxyl groups are typically carboxylated by reaction with carbon dioxide. Note the presence of two distinct Ca^{2+} binding sites

(FGF-23) as well as molecular sensors for Ca^{2+} , including the so-called calcium-sensing receptor (CaSR), and phosphate. In some cases, these sensors coordinate the synthesis and release of hormones with actions on both Ca^{2+} and phosphate transfers in targets including intestine, kidney, and bone. In other cases, they directly modulate Ca^{2+} and/or phosphate transport in key target cells. In addition, macronutrients including carbohydrates and protein are important modulators of calcium and phosphate metabolism with notable positive effects on intestinal absorption.

Introduction to Calcium and Phosphate

In serum, calcium is present in three main forms: ionized, complexed with small anions such as citrate, and bound to serum proteins, chiefly albumin. After ingestion, calcium is released from foods, in part by the action of gastric acid as well as digestive enzymes in the stomach and small

intestine. Calcium absorption is mediated by transport mechanisms in all segments of the small intestine. Even so it is inherently inefficient. Typically, significantly less than 50% of ingested calcium is absorbed (Fig. 2.2).

Phosphoric acid is a strong acid with three pH titratable hydroxyl (OH) groups. For this reason, inorganic phosphate exists in three forms: H_2PO_4^- , HPO_4^{2-} , and PO_4^{3-} . Under standard conditions at 25°C, the pKas that govern the equilibria between the species are (1) 2.13; (2) 7.20; and (3) 12.36 [1]. At physiological ionic strength and temperature, these pKa values shift so that pKa_2 , for example, falls from 7.2 to around 6.8. Thus, in blood plasma at pH 7.4 with a total inorganic phosphate concentration of around 1.0 mM, the HPO_4^{2-} concentration is approximately 0.8 mM and the H_2PO_4^- concentration is around 0.2 mM; the concentrations of other species are negligible. In the acid environment of the distal nephron, where pH values frequently drop below 5.5 as a result of proton secretion [2], H_2PO_4^- becomes the predominant species. In general, the negative

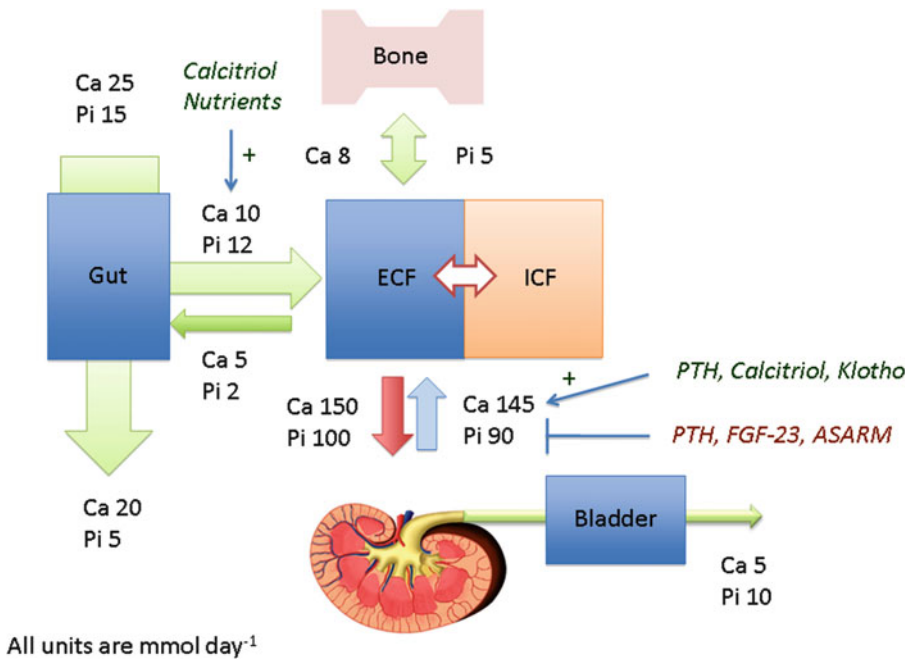


Fig. 2.2 Whole body calcium and phosphate fluxes in adult humans. Representative fluxes are shown for calcium and phosphate transfers between the extracellular fluid and the gut, kidney, and bone and have been based on previous analyses [208, 270]. The ratio of

the fluxes for calcium and phosphate between bone and the extracellular fluid reflects the composition of hydroxyapatite. The fluxes shown between the ECF and kidney refer to glomerular filtration and tubular reabsorption. ECF, extracellular fluid; ICF, intracellular fluid

charge on phosphate is balanced in body fluids by the monovalent cations Na^+ or K^+ with no implications for its water solubility. However, HPO_4^{2-} forms insoluble precipitates with Ca^{2+} ions in the presence of water with a solubility product of around $5 \times 10^{-7} \text{ (mol/L)}^2$. At the normal ionized concentrations for Ca^{2+} of 1.2 mM and HPO_4^{2-} of 0.8 mM in human plasma, this product is exceeded. Nevertheless, precipitation and bone mineral formation do not proceed spontaneously in body fluids or in the interstitium due, in part, to the presence of Mg^{2+} , which competes with Ca^{2+} [3], and forms phosphate complexes of greater solubility [4], as well as inhibitory polyphosphates and proteins (see below).

Intracellular and Extracellular Roles of Calcium and Phosphate

Ca^{2+} and inorganic phosphate play multiple physiological roles, many of which are independent of their roles in mineralization. *Intracellularly*, Ca^{2+} acts as a pluripotent regulator of biochemical pathways via high affinity interactions with rate-limiting enzymes or protein modulators. Phosphate, on the other hand, acts as a substrate for the synthesis of organic phosphates in biochemical interconversions, molecular recognition, energy storage (e.g., in ATP and creatine phosphate) or as a regulator of protein conformation.

Extracellularly, Ca^{2+} also supports synaptic transmission, the contractile state of vascular and nonvascular smooth muscle, the final common pathway of coagulation, and platelet aggregation; it also acts as a reservoir for the replenishment of intracellular Ca^{2+} pools. The requirements of all these processes have led to the establishment of a tight physiological range for the extracellular ionized Ca^{2+} concentration (1.1–1.3 mM) that is defended at the lower end by the secretory state of the four parathyroid glands under tight feedback control mediated by extracellular Ca^{2+} -sensing receptors (review: [5]) and at the higher end by renal Ca^{2+} -sensing receptors coupled to stimulated calcium excretion [6]. Calcitriol and FGF-23 modulate the parathyroid response: calcitriol by enhancing Ca^{2+} -sensing receptor expression [7] and suppressing PTH expression [8, 9];

FGF-23 by suppressing PTH gene expression [10]. Interestingly, the immediate precursor of calcitriol, 25-hydroxyvitamin D significantly suppresses PTH secretion and mRNA levels at physiologically relevant concentrations [11], suggesting either that 25-hydroxyvitamin D directly activates the parathyroid vitamin D receptor or acts as the substrate for the local generation of calcitriol.

Like Ca^{2+} , the extracellular inorganic phosphate concentration is tightly regulated. However, there is greater variation in its normal range from around 0.7 to 1.4 mM, providing variation in the Ca^{2+} -phosphate solubility product and, thus, in the potential rates of hydroxyapatite formation. Hypophosphatemia is a recognized cause of rickets and osteomalacia (review: [12]) as considered below.

An Overview of Calcium and Phosphate Metabolism

Calcium and phosphate exchange between the blood plasma and extracellular fluid as well as the following major compartments: the intestinal lumen, the intracellular fluid, bone mineral, and renal tubular fluid (Fig. 2.2). In consequence, the overall balances for calcium and phosphate are determined by the rates of intestinal absorption and secretion, the rates of renal filtration and reabsorption, the rates of uptake and extrusion from the cytoplasmic compartment of all tissues, and the rates of bone mineralization and resorption. While the rates of calcium and phosphate uptake and release from bone mineral are matched by the structure of the repeating unit of hydroxyapatite, $(\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2)$, in which the relative proportion of Ca:phosphate is 5:3, the rates of calcium and phosphate transfers between the extracellular fluid and other compartments are less predictable and regulated independently. Nevertheless, key hormonal regulators of calcium metabolism also impact on phosphate metabolism. Notable examples are PTH and, calcitriol. Major relationships between calcium, phosphate, PTH, calcitriol, and FGF-23, the key phosphate regulating hormone, as well as calcitonin are presented in Fig. 2.3 and discussed below.

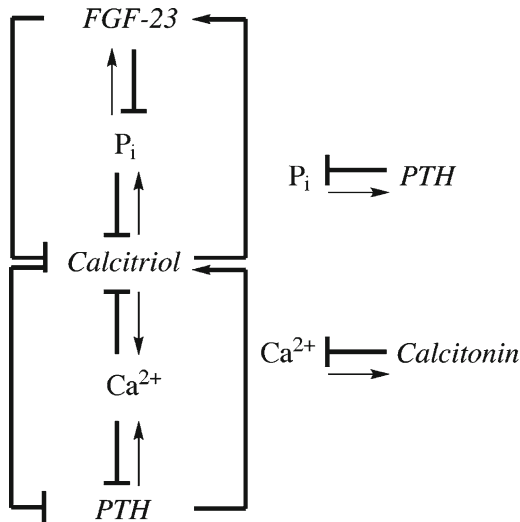


Fig. 2.3 Relationships between calcium and phosphate with key mineral-regulating hormones. The *left-hand panel* shows the relationships between calcium and phosphate with calcitriol, the key positive modulator of their intestinal absorption. In addition, the feedback relationships between calcium and PTH (*bottom*) as well as the phosphate and FGF-23 (*top*) are shown. Elevated calcium suppresses PTH secretion within minutes; hypocalcemia acutely stimulates PTH secretion. Elevated phosphate, on the other hand, promotes the production of FGF-23 only after a delay of several hours. Also shown in the *left-hand panel* are the major reciprocal relationships between PTH and calcitriol, and calcitriol and FGF-23. PTH stimulates calcitriol synthesis; calcitriol promotes the production of FGF-23, thereby reducing the risk of hyperphosphatemia following enhanced intestinal phosphate absorption. On the other hand, FGF-23 suppresses calcitriol synthesis and calcitriol suppresses PTH synthesis. These relationships may contribute to a reported positive effect of PTH on FGF-23 synthesis and a reported negative effect of FGF-23 on PTH synthesis. The *right-hand panels* show the feedback relationships between phosphate and PTH as well as calcium and calcitonin. PTH lowers the serum phosphate concentration by suppressing its reabsorption in the proximal tubule and calcitonin suppresses the serum calcium concentration by suppressing osteoclastic resorption of bone

Parathyroid Hormone

PTH secretion is under the tight inhibitory control of the serum ionized calcium (Ca^{2+}) concentration acting via the CaSR (review: [5]). Thus, PTH secretion is markedly stimulated by hypocalcemia. In addition, PTH secretion is negatively modulated by various factors including L-amino

acids [13], which enhance the extracellular Ca^{2+} sensitivity of the CaSR (reviews: [14, 15]), as well as calcitriol, which upregulates CaSR expression [7] and suppresses PTH expression (review: [16]), 25-hydroxyvitamin D [11], and FGF-23 [10]. Inorganic phosphate promotes parathyroid hyperplasia and PTH secretion and these effects appear to contribute to secondary hyperparathyroidism in chronic kidney disease [17]. Endogenous expression of the single-pass integral membrane protein, α -klotho, in the parathyroid has been reported to facilitate PTH secretion [18].

Consistent with the stimulatory impact of hypocalcemia on its secretion, key actions of PTH are directed to elevating the serum Ca^{2+} concentration and restraining or suppressing the inorganic phosphate concentration to prevent inappropriate crystallization. Thus, PTH promotes osteoclastic bone resorption via type-I PTH/PTHrP receptors (PTH1Rs) on cells of the osteoblast–osteocyte lineage as well as T lymphocytes [19]. It also modulates renal tubular transport via independent effects to stimulate Ca^{2+} reabsorption and inhibit phosphate reabsorption. Finally, it promotes 1α -hydroxylase activity in the renal proximal tubule to boost serum calcitriol levels [20] and, thus, stimulate intestinal calcium absorption. In consequence, endogenous PTH is best viewed as a surveillance and emergency-response hormone directed to the prevention and reversal of hypocalcemia ([6]; review: [21]).

PTH stimulates calcium reabsorption in the distal nephron [22]; thereby, enhancing total renal calcium reabsorption from around 95% to 98.5% and thus suppressing renal calcium excretion from around 5% to 1.5% of a largely fixed, filtered load [23]. On the other hand, PTH suppresses phosphate reabsorption in the proximal tubules [24], markedly elevating renal phosphate excretion from around 15–20% to greater than 50% of the filtered load. Correspondingly, parathyroidectomy suppresses fractional phosphate excretion to around 10% [25]. PTH-induced renal phosphate wasting would appear to be an appropriate compensatory response to calcitriol-stimulated small intestinal phosphate uptake and osteoclastic release of phosphate from resorbed hydroxyapatite in bone. Evidence that PTH [26] and, indeed, calcitriol [27]

promote the production of the phosphatonin FGF-23 in bone provides additional mechanisms for the activation of renal phosphate wasting under conditions of PTH-stimulated bone resorption and calcitriol synthesis.

Although prolonged elevations in PTH induce osteoclastic bone resorption, acute administration of PTH, in pharmacological doses and in the context of normal serum calcium levels, promotes bone formation (review: [28]), possibly simulating some of the effects of bone cell-derived PTHrP (review: [29]). Thus, acute repetitive stimulation of PTH1Rs on osteoblasts and/or osteocytes for 1–2 h promotes bone formation and mineralization. Perhaps consistent with this, the immediate response to acute administration of PTH is a *fall* rather than a rise in serum Ca^{2+} concentration associated with calcium uptake by bone [30, 31].

The outcome of PTH action is, thus, dependent upon context. The duration of exposure, the serum level of calcitriol and its precursor 25-hydroxyvitamin D, the dietary intakes and serum levels of calcium and phosphate, together with the relative levels of the responses in bone and kidney all contribute to the outcome. In the context of prolonged elevation of PTH, the serum calcium concentration rises because the effects of PTH, whether direct or indirect, on all key targets promote Ca^{2+} accumulation in the extracellular fluid whether from bone, kidney, or gut. However, the impact of prolonged elevation of PTH on phosphate is less predictable and depends on whether the primary effect is on the kidney, in which case serum phosphate levels may drop or the gut, via calcitriol, or on bone in which case serum phosphate levels are more likely to be normal. In the setting of chronic kidney disease, in which there is resistance to PTH actions, serum phosphate levels are typically elevated and serum calcium levels are normal or suppressed (review: [32]).

Calcitriol and 25-Hydroxyvitamin D

Calcitriol is synthesized from 25-hydroxyvitamin D by the action of 1α -hydroxylase, the product of the *CYP27B1* gene (review: [33]). In serum,

calcitriol is a tightly regulated hormone arising from the actions of multiple nutrient and hormonal inputs on 1α -hydroxylase expression in the renal proximal tubules. Its primary actions are to promote intestinal calcium and phosphate absorption via effects on duodenal and jejunal enterocytes, respectively (review: [33]) and failure of these effects can be overcome by diets that are rich in calcium, phosphate, and certain macronutrients including lactose and protein. Its secondary actions include enhanced renal reabsorption of calcium (review: [33]) and, possibly, phosphate [34]. Together, enhanced absorptions of calcium and phosphate ions are detected by local Ca^{2+} and phosphate sensors in the gastrointestinal tract and, if sufficient to elevate the serum calcium and/or phosphate levels, by sensors strategically positioned in endocrine, bone, and kidney cells. In this way, enhanced calcium absorption, whether arising from calcitriol-stimulated vitamin D receptors (VDRs) in the duodenum or via an alternative small intestinal absorption pathway can shut down PTH secretion via extracellular Ca^{2+} -sensing receptors on parathyroid cells (review: [5]). Together with the elevated serum calcium and phosphate levels, suppressed PTH levels remove an important stimulatory drive for renal calcitriol synthesis (review: [33]).

In company with adequate levels of serum calcium, calcitriol also suppresses the growth of parathyroid glands in 1α -hydroxylase null mice (review: [35]). Nevertheless, the normal physiological significance of these effects is unclear. In part, this uncertainty arises from observations of a lack of correlation between the serum calcitriol and PTH levels in human subjects, and from the primacy of the serum Ca^{2+} concentration in determining serum PTH levels.

25-Hydroxyvitamin D is both the substrate for the synthesis of calcitriol and a nutritional modulator in its own right. With respect to mineral metabolism, its primary target appears to be the parathyroid where, in concert with the serum Ca^{2+} concentration, it suppresses PTH synthesis. This explains highly reproducible negative correlations between serum 25-hydroxyvitamin D and PTH levels (review: [36]) and is supported by evidence that 25-hydroxyvitamin D suppresses

PTH gene expression and secretion from cultured parathyroid cells in both the absence and presence of a 1α -hydroxylase inhibitor [11]. Furthermore, in *CYP27B1* null mice, vitamin D3 supplements sufficient to raise the serum 25-hydroxyvitamin D level to 400 nmol/L prevented hypocalcemia and markedly increased the expressions of the VDR-sensitive targets, intestinal calbindin- D_{9k} , and renal 24-hydroxylase on a standard diet [37]. Whether these effects operate at more commonly observed concentrations in humans (around 30–80 nmol/L) is currently unclear.

The significance of serum calcitriol levels and calcitriol-dependent activation of small intestinal VDRs for whole body mineral metabolism depends on dietary context. It is critical under conditions of low calcium and/or phosphate intake and in the absence of dietary lactose upon weaning in mice (review: [33]). Under these circumstances, 1α -hydroxylase null or VDR null mice develop hypocalcemia, hypophosphatemia, and secondary hyperparathyroidism taking the form of massively elevated serum PTH levels [38, 39]. However, calcium and phosphate absorption is restored, as reported by serum calcium, phosphate, and PTH levels, upon the introduction of a diet containing 2% calcium, 1–2% phosphate, and 20% lactose [40]. These findings are consistent with the notion of a calcitriol-independent absorption pathway linked to macronutrient digestion and absorption (see below). Under conditions in which dietary calcium and phosphate intake is high and intestinal absorption is supported by adequate levels of key macronutrients, as may frequently occur in humans, the impact of serum calcitriol on mineral metabolism may be limited, as extracellular Ca^{2+} -dependent suppression of PTH removes a key drive for renal calcitriol synthesis. Under these conditions, there is no correlation between serum PTH and calcitriol levels and the negative impact of exogenously administered calcitriol on PTH synthesis and secretion (review: [41]) may be primarily of pharmacological significance. Nevertheless, under these conditions, serum 25-hydroxyvitamin D retains significance as a nutritional modulator with the ability to suppress PTH whether alone or in concert with calcium [42].

As bodily vitamin D stores fall in humans whether due to inadequate diet or intestinal absorption, poor sunlight exposure or skin biosynthesis, or a combination of these factors, as frequently occurs in the elderly (review: [43]), serum 25-hydroxyvitamin D levels fall with attendant increases in serum PTH levels. The serum calcium concentration typically remains in the normal range. Whether elevated serum PTH levels act to maintain or boost serum calcitriol levels under these circumstances is unclear—typically, serum calcitriol levels also remain within the normal reference range. However, there is a cost: accelerated consumption of 25-hydroxyvitamin D. Oral calcium supplements can facilitate calcium absorption in the context of vitamin D deficiency and thereby boost serum ionized Ca^{2+} levels, typically, still within the normal range. This provides Ca^{2+} -dependent feedback inhibition of renal calcitriol synthesis as well as PTH secretion, further relieving the drive for calcitriol synthesis [44]. Conversely, repletion of vitamin D stores is reflected first in elevated 25-hydroxyvitamin D levels with corresponding falls in serum PTH [45].

25-Hydroxyvitamin D is converted to the key calcium and phosphate-elevating hormone, calcitriol via the action of 1α -hydroxylase. Although the gene *CYP27B1* appears to universally encode 1α -hydroxylase in tissues including the kidney, testis, brain, placenta, bone, and skin, thereby catalyzing the local production of calcitriol (review: [46]), only the renal enzyme controls the synthesis of calcitriol for release into the circulation under physiological conditions and is selectively controlled by key micronutrient and hormonal regulators of mineral metabolism (review: [33]). These regulators include elevations in the serum levels of calcium or phosphate (negative), as well as the hormones FGF-23 and calcitriol (also negative), and PTH and IGF-1 (both positive). Downregulation of renal 1α -hydroxylase by calcium, phosphate, FGF-23 (as a delayed reporter of ingested phosphate), and calcitriol itself may all be viewed as physiologically appropriate forms of negative feedback. On the other hand, upregulation of 1α -hydroxylase by PTH and IGF-1 would appear to arise in quite different physiological contexts with

distinct implications. Thus, upregulation of 1α -hydroxylase by PTH may be viewed as a component of an “emergency response to hypocalcemia,” whose goal is the restoration of normal serum calcium levels. Upregulation of 1α -hydroxylase by IGF-1, on the other hand, would appear to provide a mechanism by which intestinal calcium and phosphate absorption can be enhanced together with their renal retention in the context of growth and an associated stimulation of bone formation [47–49].

Together, 25-hydroxyvitamin D and calcitriol are carried in the circulation by a 52-kDa vitamin D binding protein (DBP) and both are deactivated by a widely expressed 24-hydroxylase, encoded by the *CYP24A1* gene, whose expression is powerfully upregulated by calcitriol [50, 51] and possibly 25-hydroxyvitamin D [52] leading to the generation of 24,25(OH)₂ vitamin D or 1α -hydroxy-23-carboxy-24, 25, 26, 27-tetranorvitamin D₃ (calcitric acid). Based on findings in *CYP24a1* null mice, 24-hydroxylase prevents inappropriate elevation of serum calcitriol levels as well as attendant hypercalcemia and hypoparathyroidism [53].

In the renal synthesis of calcitriol, the DBP–25-hydroxyvitamin D complex enters the proximal tubular cytoplasm following glomerular filtration and uptake via apical megalin/cubulin-mediated endocytosis [54–56]. DBP null mice exhibit markedly suppressed levels of circulating calcitriol and 25-hydroxyvitamin D, and a demineralizing phenotype [57]. Surprisingly, serum calcium, phosphate, and PTH levels were normal in DBP null mice on control chow but administration of a vitamin D-deficient diet for 4 weeks induced a significant decrease in serum phosphate and a significant increase in serum PTH without a change in serum calcium [57] indicating that the elevated PTH had normalized serum calcium in the context of vitamin D deficiency while suppressing the serum phosphate level. Whether serum FGF-23 levels changed under the conditions of these experiments is unknown. The results indicate that DBP provides a store of circulating vitamin D metabolites and prolongs their serum half-lives but is not necessary for their actions (review: [58]).

The significance of calcitriol for normal mineral homeostasis has been assessed in 1α -hydroxylase

(*CYP27B1*) null mice and VDR null mice. Although the phenotypes of these mice are not identical suggesting the existence of calcitriol-independent actions of VDR in the skin and hair follicles, and VDR-independent actions of calcitriol in the epiphyseal growth plates (review: [33]), they both exhibit severe rickets and osteomalacia after weaning, arising from markedly impaired intestinal calcium and phosphate absorption. The findings indicate that, postweaning, adequate circulating levels of calcitriol together with the expression of its cognate VDRs in the small intestine are required for normal mineral metabolism (review: [33]). Thus, intestinal calcium and phosphate transport was restored in *CYP27B1* null mice following administration of exogenous calcitriol [59], and in global VDR null mice by intestine-specific transgenic expression of the VDR [60]. Furthermore, the hypo-mineralizing bone phenotype was clearly dependent upon impaired intestinal calcium and phosphate absorption because it could be overcome by rescue diets containing enhanced contents of calcium and phosphate as well as lactose (review: [33]). The expression of the phenotype only after weaning and the striking impact of lactose in the rescue diet suggests the existence of a calcitriol-independent, macronutrient-associated absorption pathway (see below).

FGF-23 and Klotho

FGF-23 is a “phosphatonin,” i.e., a circulating factor that promotes renal proximal tubular phosphate wasting and thereby lowers serum phosphate levels (reviews: [61, 62]). Although its impact on phosphate metabolism in certain pathological contexts seems clear, its role in the normal surveillance of bodily phosphate metabolism and the time-scale over which it operates are uncertain.

The significance of FGF-23 for the control of renal phosphate excretion and serum phosphate levels was first established in human studies of inherited forms of rickets and/or osteomalacia including autosomal dominant hypophosphatemic rickets (ADHR) [63] and X-linked hypophosphatemia (XLH) [64] as well as tumor-induced osteomalacia [64] in all of which serum FGF-23

levels are elevated in association with renal phosphate wasting, hypophosphatemia, and inappropriately suppressed serum calcitriol levels (review: [41]). Correspondingly, tumoral calcinosis in humans in the context of hyperphosphatemia and elevated serum calcitriol levels typically arises from impaired FGF-23 secretion whether associated with specific FGF-23 mutations [65] or mutations of UDP-GalNAc transferase 3 (GALNT3) that impair FGF-23 processing (review: [66]).

The significance of FGF-23 in the control of phosphate metabolism is supported by transgenic mouse studies, most notably *fgf-23* null mice, which exhibit hyperphosphatemia, elevated serum calcitriol levels, hypercalcemia, and widespread calcification [67]. Elevated serum calcitriol levels appear to be critical for the development of both hyperphosphatemia and hypercalcemia in these mice. Consistent with this notion, the double mutant *fgf23^{-/-}/1 α -OHase^{-/-}* mouse exhibits normal or near-normal serum calcium and phosphate levels as well as normal growth and markedly improved lifespan [68].

FGF-23 is a key negative regulator of serum calcitriol levels. Thus, an acute bolus intravenous injection of FGF-23 in mice suppressed 1 α -OHase expression and promoted 24-hydroxylase expression, resulting in markedly suppressed serum calcitriol levels [69]. The effect of FGF-23 on serum calcitriol levels was detected between 3 and 24 h (trough around 9 h) indicating that its effects are likely to be dependent on changes in gene expression. Interestingly, the effect of FGF-23 on the serum calcitriol level in these studies occurred several hours earlier, and was substantially greater, than its effect on the serum phosphate level [69]. Thus, although FGF-23 promotes phosphaturia by downregulating the expressions of Npt2a and Npt2c in the renal proximal tubular luminal membrane [70], and suppresses serum phosphate levels, it is not yet clear whether its effects are mediated directly by FGF receptors in the renal proximal tubule or indirectly via other hormonal factors, e.g., arising from the processing of small integrin-binding ligand N-linked glycoproteins (SIBLINGs) to acidic serine and aspartate-rich motif (ASARM) peptides in bone or via paracrine factors, e.g., from the distal tubule (review: [71]).

Complicating these considerations is an observation that FGF-23 suppresses intestinal Npt2b expression in wild-type but not Npt2a or Npt2c null mice [72] independent of serum calcitriol levels, which were markedly suppressed in all three cases. The results point to the existence of, as yet unrecognized, phosphate regulatory signals that operate between the kidney and small intestine and are mediated by Npt2a and 2c and/or one of their partner proteins.

The pronounced negative effect of serum FGF-23 on serum calcitriol levels raised the possibility of a novel feedback loop involving the two hormones. Consistent with this idea, calcitriol activates FGF-23 expression [27, 73, 74]. In addition, prolonged elevations of calcitriol upregulate the expression of FGF-23 and prolonged elevations of FGF-23 coordinately downregulate renal 1 α -hydroxylase and upregulate renal 24-hydroxylase (review: [41]). The feedback loop between calcitriol and FGF-23 may contribute to the support of a natural diurnal rhythm in whole body calcium and phosphate metabolism, which in part takes the form of a coordinated trough in the serum levels of calcium (-2%), phosphate (-10%), calcitriol (-10%), and PTH (-20%) at around 0800 [75]. In a recent study, no diurnal rhythm was observed in serum FGF-23 levels, however, there was a pronounced trough in the level of the soluble form of its co-receptor α -klotho (-40%) at around 0000 [76]. The significance of these findings is currently unclear.

FGF-23 is synthesized in osteocytes and osteoblasts and its secretion depends, in part, on the enzyme GALNT3 which O-glycosylates the peptide (review: [41]). Its expression and serum levels are increased by elevated dietary phosphate intake [77–79] and elevated serum calcitriol levels [27] and these effects have been demonstrated in both mouse and human studies. However, serum FGF-23 levels do not appear to be regulated by acute changes in serum phosphate concentration. Data obtained in acute human studies, for example, suggest that serum FGF-23 levels do not normally respond within 6 h of exposure to a substantial increase in either dietary phosphate intake or the serum phosphate level [79, 80] and possibly not before 12 h [81].

PTH has been reported to promote FGF-23 expression and elevate its serum levels. Thus, PTH administered subcutaneously in mice for 3 days by osmotic mini-pump induced an elevation in serum FGF-23 levels in the context of elevated serum calcium and suppressed serum phosphate levels [26] and in a study in healthy men who received 18-h intravenous infusions of PTH 1–34, serum FGF-23 levels were elevated from 6 to 18 h in association with elevated serum levels of calcitriol and calcium [82]. Whether reported reciprocal interactions between PTH and FGF-23 [10, 26] constitute an additional regulatory loop or are dependent upon the intermediary actions of calcitriol and/or phosphate is currently uncertain.

α -Klotho

α -Klotho is an important partner of FGF-23 and exists in both circulating and integral membrane forms. It contributes to the formation of high affinity FGF-23 receptors by forming ternary complexes with FGF receptors [83] including FGFR1c in the proximal tubule [70]. Consistent with the notion that the actions of α -klotho and FGF-23 are interdependent, the phenotypes of α -klotho null mice and FGF-23 null mice are similar and both include marked elevations of serum phosphate and increased serum levels of calcitriol [84]. In addition, FGF-23 levels are markedly elevated in α -klotho null mice [85] suggesting the existence of a resistance state with dysregulated FGF23 secretion arising from elevated serum calcitriol and/or serum phosphate levels.

More recent work suggests that α -klotho may have additional actions on mineral metabolism that arise, at least in part, from its β -glucuronidase activity. Thus, α -klotho promotes the surface expression of distal nephron TRPV5 [86] by cleaving terminal sialic acid residues to expose an underlying galactose-*N*-acetylglucosamine, which binds to the plasma membrane lectin, galectin-1 [87]. The outcome is enhanced renal Ca^{2+} reabsorption (review: [88]). Consistent with these findings the α -klotho null mouse exhibits impaired

distal convoluted tubule (DCT) and connecting tubule Ca^{2+} reabsorption and resistance to the stimulatory action of PTH on Ca^{2+} reabsorption [89]. In addition, as noted above, α -klotho expression in the parathyroid has also been linked to PTH secretion [18].

Calcium-Sensing Mechanisms: Calcium-Sensing Receptors

Although a number of cellular calcium-sensing mechanisms have been defined based on the activities of ion channels and transporters, one of the best understood calcium-sensing mechanism in human biology is dependent upon the expression of a class C G-protein-coupled receptor known as the CaSR. The CaSR was first cloned from the parathyroid [90], where it mediates feedback control of PTH secretion and thus provides a key defense against hypocalcemia (review: [5]) and subsequently the kidney [91], where it regulates renal calcium excretion, providing an important defense against hypercalcemia [6]. The CaSR also modulates renal phosphate, salt, and water transport (review: [92]). It is expressed in various bodily compartments and organs including the gastro-intestinal tract, kidney, thyroid C-cells, and bone in which it is expressed on cells of both the osteoblast and osteoclast lineages [93]. The roles of the CaSR in the control of renal calcium and phosphate transport as well as bone biology are considered below. In gut biology it has multiple roles that include the promotion of interactions between macronutrient and micronutrient metabolism (review: [94]).

Heterozygous inactivating mutations of the CaSR underlie the uncommon, typically benign, hypercalcemic disorder known as familial hypocalciuric hypercalcemia (FHH) [95] in which there is impaired inhibition of calcium reabsorption in the renal cortical thick ascending limb (TAL) [96]. The primary problem is impaired inhibitory control of renal calcium reabsorption but, in addition, the serum PTH level is typically inappropriately normal [97]. The rare, typically homozygous, form of the disorder presents as a

neonatal form of severe hyperparathyroidism with markedly elevated serum calcium levels and pathological fractures within the first weeks of life [98]. The problem arises from extreme resistance to the serum Ca^{2+} concentration in both the parathyroid and kidney.

In addition to sensing the extracellular Ca^{2+} concentration, the CaSR is a multimodal chemosensor that responds to L-amino acids and is, thus, sensitive to changes in protein nutrition (review: [99]), as well as ionic strength, pH, Mg^{2+} , neuromodulators including spermine, and glutathione analogs (reviews: [15, 100]). Thus, the CaSR has roles not only in the regulation and modulation of calcium and phosphate metabolism and bone biology but also in various other physiological processes. In the gastro-intestinal tract, these include the modulation of gastrin release and acid secretion (review: [99]) as well as the regulation of cholecystokinin secretion [101–103]. The CaSR may also contribute to well-recognized positive interactions between protein nutrition and calcium and bone metabolism [104].

Phosphate-Sensing Mechanisms

Phosphate-sensing mechanisms have been identified in the intestine, kidney, bone, and growth plate and act to regulate renal phosphate excretion and skeletal development as well as bone mass and mineralization (see below). Biological effects coupled to phosphate-sensing mechanisms include enhanced production of phosphatonins including FGF-23, and MEPE, and suppressed production of calcitriol, both of which are appropriate responses to hyperphosphatemia, as well as apoptosis of hypertrophic chondrocytes in the process of normal growth plate development and osteoblast maturation (review: [105]). In addition, a number of putative phosphate sensors, e.g., based on one or more phosphate transporters, and even elements of potential signaling pathways, e.g., ERK1/2, have been proposed [106]. However, the full significance of these pathways awaits the identification of the molecular sensors and their signaling partners.

Calcium Transport Mechanisms

Epithelial Calcium Transporters in Support of Transcellular Ca^{2+} Transport

Expression cloning of key epithelial Ca^{2+} transporters of the small intestine [107] and kidney [108], permitted the identification and detailed analyses of active transport mechanisms in the duodenum and distal nephron (review: [109]). These transporters are now recognized as members of the TRPV family of ion channels, i.e., TRPV6 and TRPV5, respectively (review: [110]). In support of these transport mechanisms are the cytoplasmic Ca^{2+} -binding proteins calbindin- D_{9k} (intestine and kidney) and calbindin- D_{28k} (kidney), which promote calcium uptake and transport by buffering the intracellular Ca^{2+} concentration, as well as two energy-expending basolateral Ca^{2+} pump mechanisms: the Ca^{2+} -ATPase isoform Ib and the $\text{Na}^+/\text{Ca}^{2+}$ -exchanger NCX1 coupled to Na^+/K^+ -ATPase activity (review: [109, 110]). The Na^+/K^+ -ATPase maintains a steep electrochemical gradient for Na^+ ions across the basolateral membrane favoring Na^+ influx from the extracellular fluid to the cytoplasm. The Na^+ gradient is used by NCX1 to drive Ca^{2+} efflux in exchange for Na^+ influx (Fig. 2.4; upper cell). These mechanisms support calcitriol-induced Ca^{2+} absorption in the duodenum (review: [110]) and PTH and calcitriol-induced Ca^{2+} reabsorption in the distal nephron (review: [22]).

In addition, less well-defined transcellular Ca^{2+} transport mechanisms provide a link between calcium absorption and the absorption of key macronutrients (see below).

Paracellular Ca^{2+} Transport Across Epithelia

In addition to transcellular mechanisms of calcium transport, paracellular Ca^{2+} transport mechanisms support Ca^{2+} transport in both the intestine and renal tubules. The key elements of these transport mechanisms are a transepithelial electrochemical gradient and a paracellular pathway (Fig. 2.5). A favorable transepithelial

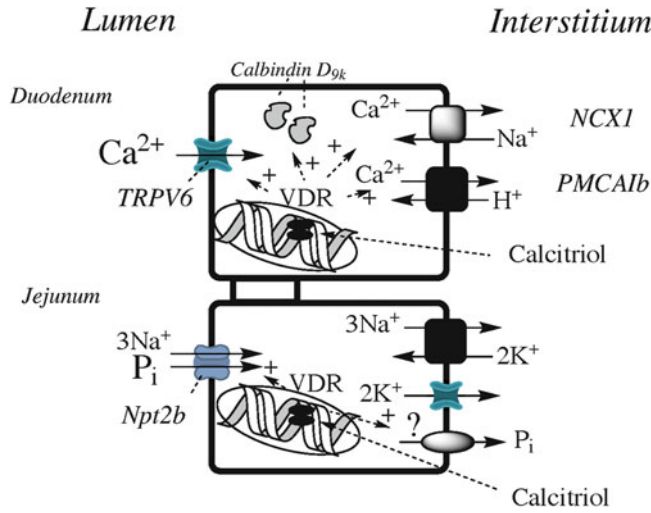


Fig. 2.4 Calcitriol stimulated intestinal absorption pathways for calcium and phosphate. The *upper cell* presents components of a key duodenal calcium absorptive pathway including the luminal membrane Ca²⁺ channel, TRPV6, the intracellular Ca²⁺ binding protein, calbindin D_{9k}, which buffers the cytoplasmic Ca²⁺ concentration, as well as exit pathways via the Na⁺/Ca²⁺ exchanger, NCX1 and the Ca²⁺-ATPase, PMCA1b (review: [110]). The basolateral Na⁺/K⁺-ATPase, which maintains the Na⁺ gradient across the interstitium-facing membrane is not shown.

The *lower cell* presents components of a key jejunal phosphate absorptive pathway (review: [114]), including the luminal membrane Na⁺-phosphate co-transporter, Npt2b, basolateral Na⁺/K⁺-ATPase, and a basolateral membrane phosphate channel (or transporter). Calcitriol acts via nuclear vitamin D receptors to upregulate the synthesis of molecular components in both pathways. Additional pathways for the small intestinal absorption of calcium, and possibly phosphate, are linked to macronutrient uptake (not shown)

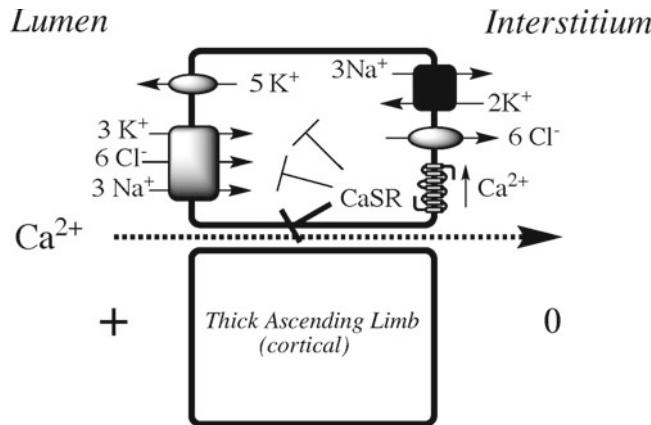


Fig. 2.5 Inhibitory control of paracellular calcium reabsorption in the cortical thick ascending limb by calcium-sensing receptors. Basolateral calcium-sensing receptors are activated by elevated extracellular fluid Ca²⁺ concentration, e.g., in the context of systemic hypercalcemia or

in response to transient local hyper-reabsorption (review: [96]). A signaling pathway linked, in part, to the production of arachidonic acid metabolites suppresses either the transepithelial driving force for reabsorption or Ca²⁺ permeation via the intercellular junctions

electrochemical gradient may arise from either a lumen positive potential difference (expressed with respect to a reference electrode in the interstitium) and/or a transepithelial Ca^{2+} concentration gradient that favors transport. In addition, paracellular transport requires the presence of a cation-selective intercellular pathway via the junctional complexes. The cloning and identification of claudins, cation-selective proteins that are expressed in tight junctional complexes (review: [111]) have greatly enhanced understandings of these mechanisms and the nature of their regulation. Key sites for paracellular calcium transport include the renal proximal tubule and TAL of Henle's loop (see below).

Inorganic Phosphate Transport Mechanisms

Inorganic Phosphate Transporters

Three families of inorganic phosphate transporters have been identified: so-called type-I transporters of the SLC17 family, which are now recognized as nonselective organic anion transporters (review: [112]); type-II transporters of the SLC34 family, including NaPi-IIa, -IIb, and -IIc, also known as Npt2a, 2b, and 2c, respectively, which are Na^+ -dependent, selective for the divalent HPO_4^{2-} species and expressed in the brush border (luminal) membranes of the renal proximal tubules (2a and 2c) and small intestine (2b); and type-III transporters of the SLC20 family, which are widely expressed, Na^+ -dependent, selective for the monovalent H_2PO_4^- species and include PiT-1 and PiT-2 (reviews: [113, 114]).

Despite the identification of transporters on the luminal brush border membranes of transporting epithelia such as small intestine and renal proximal tubule, the identities of phosphate transporters on the basolateral membranes, which are required to deliver inorganic phosphate to the blood, are unclear. Based on energetic considerations, which include a cell-negative membrane potential with respect to the extracellular fluid, and an intracellular inorganic phosphate concentration

that is likely to be elevated with respect to the extracellular fluid, phosphate transport across epithelial basolateral membranes is likely to be passive and, thus, mediated by (1) phosphate-permeant anion channels, (2) an electroneutral transporter such as the Band-3 anion exchanger of erythrocytes [115], or (3) a phosphate carrier analogous to mitochondrial SLC25A3 [116].

Intestinal Calcium Absorption

Intestinal calcium absorption is largely confined to the small intestine and is notably inefficient. Thus, despite the existence of various mechanisms to promote absorption, absorption rates are typically less than 50% of ingested loads (Fig. 2.2). Macronutrients including protein [117, 118] and carbohydrates such as glucose [119] and lactose [40, 120] promote calcium absorption. In addition, calcium absorption is powerfully stimulated by calcitriol, which together with the vitamin D receptor is required to prevent hypocalcemia and secondary hyperparathyroidism in mice after, but not prior to, weaning (review: [33]). Intestinal calcium absorption is positively modulated by estrogens [121–123], as well as prolactin [124, 125] and placental lactogen [126]. Consistent with these effects, intestinal calcium absorption is impaired in vitamin D deficiency (review: [127]), reduced in postmenopausal women (review: [128]), and enhanced in pregnancy [129]. The molecular bases for the effects of calcitriol, estrogens, and prolactin/placental lactogen are not yet clear. Nevertheless, calcitriol and estrogens (reviews: [109, 110]) as well as prolactin [130] upregulate several recognized molecular components of Ca^{2+} transport.

Ca^{2+} ions are absorbed from all three major segments of the small intestine: the duodenum; the jejunum; and the ileum. However, the mechanisms of calcium absorption differ between these segments. In particular, active calcium absorption localizes to the duodenum [131] where it is stimulated by calcitriol [132] and calcium absorption that is coupled to the absorption of glucose or lactose is primarily located in the jejunum and

ileum [133, 134]. The location and molecular basis of protein-linked Ca^{2+} absorption are currently unknown.

Several distinct transepithelial transport pathways support calcium absorption. One of the better recognized pathways operates in the duodenum and comprises: *TRPV6* Ca^{2+} channels, which mediate Ca^{2+} uptake from the intestinal lumen across the enterocyte luminal brush border membrane [107]; *calbindin-D_{9k}*, an intracellular Ca^{2+} -binding protein, which buffers the cytoplasmic ionized Ca^{2+} concentration and promotes transcellular Ca^{2+} transport; and ATP-dependent Ca^{2+} efflux into the extracellular fluid, including (1) the $\text{Na}^+/\text{Ca}^{2+}$ exchanger NCX1, coupled to Na^+/K^+ -ATPase activity, or (2) the plasma membrane Ca^{2+} -ATPase, PMCA1b (reviews: [109, 110]).

Calcitriol-Dependent Activation of Calcium Absorption

As noted above, the primary action of calcitriol is to stimulate duodenal calcium absorption. The significance of the pathway is underscored by the phenotypes of *CYP27B1* null mice, which lack 1α -hydroxylase required for calcitriol synthesis, as well as VDR null mice, which together exhibit very similar phenotypes including impaired intestinal calcium absorption, hypocalcemia, and rickets (review: [33]). Furthermore, in global VDR null mice intestine-specific expression of VDR normalized intestinal calcium absorption, serum calcium levels, and the bone phenotype [60]. Calcitriol promotes the intestinal expression of key components of duodenal Ca^{2+} transport including TRPV6, its isoform TRPV5, which is normally expressed at high levels in the kidney, calbindin- D_{9k} , and PMCA1b inviting the conclusion that calcitriol action is wholly dependent upon the upregulation of a duodenal transport “system” based on these components (reviews: [109, 110]). The key components of this transport pathway are presented in Fig. 2.4 (upper cell). Surprisingly, however, recent studies in transgenic mice indicate that the molecular basis of calcitriol action is more complicated. In particular, calcitriol-stimulated intestinal calcium

absorption was intact in calbindin- D_{9k} null mice [135] and, indeed, TRPV6 null mice [136]. Furthermore, active calcium absorption was only partially impaired in TRPV6/calbindin-9k double null mice [137]. These results suggest the existence of redundancy in the actions of calcitriol on intestinal calcium transport, e.g., via upregulation of TRPV5 in compensation for TRPV6, or the existence of an alternative pathway. Consistent with the latter notion, recent evidence suggests that calcitriol promotes paracellular calcium transport in the small intestine by modifying the expression of epithelial junction proteins including upregulation of claudins 2 and 12, which support the formation of a divalent cation-permeable pathway [138].

Macronutrient-Dependent Stimulation of Intestinal Calcium Absorption: Carbohydrates

Although vitamin D deficiency or defective VDR status promotes the development of hypocalcemia and secondary hyperparathyroidism, as well as rickets or osteomalacia (review: [33]), rescue diets with increased contents of calcium and phosphate and containing lactose [120] or glucose [119, 134] can prevent and/or restore normal calcium metabolism and the bone phenotype. In addition, the duodenal (i.e., vitamin D-dependent) contribution to Ca^{2+} absorption appears to be minor, perhaps as low as 15% of the total, when there are plentiful supplies of calcium, phosphate, and macronutrients [134].

Recent work suggests a distinct segmental pattern for macronutrient-dependent calcium absorption based on the jejunum and ileum, and an alternative molecular basis for macronutrient-linked calcium absorption. According to one hypothesis, calcium absorption in the rat jejunum and ileum is dependent upon the expression of $\text{Ca}_{v1.3}$ voltage-operated Ca^{2+} channels in the enterocyte brush-border membrane together with SGLT1 Na^+ -dependent glucose transporters [119]. SGLT1 mediates membrane depolarization secondary to Na^+ influx as well as glucose transport. Depolarization activates $\text{Ca}_{v1.3}$ chan-

nels to provide a pathway for Ca^{2+} influx [119]. Thus, the proposed mechanism couples the transports of glucose and Ca^{2+} .

Macronutrient-Dependent Stimulation of Intestinal Calcium Absorption: Protein

The intake of dietary protein, like that of carbohydrates, promotes intestinal calcium absorption [117, 118, 139] and may contribute to the recognized positive impact of dietary protein on bone health (review: [104]). The effect occurs independent of gastric acid production and attendant solubilization of Ca^{2+} salts, at least in healthy young adults [140]. Whether the effect of dietary protein localizes to a particular intestinal segment and depends on a transcellular or paracellular mechanism is not yet clear. Gaffney-Stomberg et al. demonstrated enhanced Ca^{2+} uptake into duodenal brush-border membrane vesicles prepared from female rats fed a high (40%) protein diet when compared with vesicles prepared from rats fed a low (5%) protein diet, suggesting changes in the expression of key Ca^{2+} transport proteins [139]. These results suggest the existence of a dietary protein-modulated transcellular pathway for Ca^{2+} absorption in the small intestine.

Intestinal Phosphate Absorption

The small intestine is also the primary site of inorganic phosphate absorption. Since organic as well as inorganic phosphate is ingested in the diet, the stomach provides an important site for the nonenzymatic hydrolysis of organic phosphates such as nucleotides and sugar phosphates including phytates as well as inorganic polyphosphates including pyrophosphate, octa-calcium phosphate, and hydroxyapatite. Studies in humans and rats indicate that inorganic phosphate absorption occurs throughout the small intestine, primarily localizes to the duodenum (pH 6–7) and jejunum (pH 7–8) and is mediated by the Na^+ -dependent type-IIb (Npt2b) and PiT-1 or PiT-2 transporters (review: [114]); on the other

hand, inorganic phosphate transport in the mouse primarily localizes to the ileum.

Like intestinal calcium absorption, intestinal phosphate absorption is mediated by all three segments of the small intestine. However, as noted above, important species-related differences have been identified between humans and rats on the one hand, in which phosphate transport is highest in the duodenum, and mice on the other, in which it is highest in the ileum [141]. Like intestinal calcium absorption, intestinal phosphate absorption is stimulated by calcitriol ([142]; review: [143]). Surprisingly, however, at variance with the situation for calcium absorption, the jejunum rather than the duodenum is the primary site of calcitriol-dependent phosphate transport in all three species [141]. In addition, intestinal phosphate transport is markedly enhanced by low dietary phosphate (review: [144]). Whether macronutrients promote inorganic phosphate absorption as observed for calcium absorption is unknown.

Intestinal phosphate transport is mediated by Na^+ -dependent transcellular as well as Na^+ -independent, possibly paracellular, mechanisms [145]. However, the Na^+ -dependent mechanisms are better understood and appear to mediate the effect of calcitriol. Na^+ -dependent transport is mediated primarily by Npt2b transporters and, to a lesser extent, type-III transporters including PiT-1 and PiT-2 expressed in luminal brush-border membranes (review: [114]). Interestingly, calcitriol upregulates small intestinal expression of Npt2b [141] and PiT-2 [146]. In addition, low dietary phosphate upregulates Npt2b expression [147] independent of calcitriol synthesis or the VDR [148]. Key components of the calcitriol-stimulated Npt2b pathway of intestinal phosphate transport are presented in Fig. 2.4 (lower cell).

Other Modulators of Phosphate Transport

Phosphate transport is also positively modulated by estrogens, which upregulate Npt2b expression [149] consistent with the notion that postmenopausal bone loss may arise from reduced absorp-

tion of phosphate as well as calcium (review: [150]). Other hormonal influences on dietary phosphate absorption include epidermal growth factor (EGF), which promotes Npt2b expression [151] as well as glucocorticoids [152, 153] and TNF- α [154], which suppress Npt2b expression and phosphate absorption, suggesting potential explanations of the low bone mass phenotypes associated with prolonged high-dose steroids and inflammatory bowel disease. Other negative influences on intestinal phosphate transport include advancing age as well as matrix extracellular phosphoglycoprotein (MEPE) and FGF-23 (review: [114]).

Renal Mechanisms in the Control of Calcium and Phosphate Transport

Renal Tubular Calcium Transport

Renal Ca²⁺ reabsorption occurs in the proximal tubule, medullary and cortical TAL, the DCT and connecting tubules (reviews: [96, 155, 156]). As a result, renal calcium excretion in adults typically varies from 2 to 8 mmol/day (around 1.5–5% of the filtered load). The mechanisms that support renal Ca²⁺ reabsorption differ between tubular segments and include both transcellular and paracellular mechanisms. In addition, they are differentially regulated by hormonal and nutrient-dependent control mechanisms. The primary hormonal regulator of renal Ca²⁺ reabsorption is PTH, which promotes Ca²⁺ reabsorption in the distal nephron (i.e., distinct from its proximal tubule site of action on phosphate transport). Other hormonal modulators include calcitriol (review: [157]) and calcitonin [158], both of which promote Ca²⁺ reabsorption.

Ca²⁺ Reabsorption Mechanisms in the Proximal Tubule and Thick Ascending Limb

The cellular basis of Ca²⁺ reabsorption in the proximal tubule and TAL is predominantly paracellular, i.e., via the tight junctions between

neighboring cells (review: [155]). It is, thus, governed by the transepithelial electrochemical gradient as well as the ion selectivity and conductance of the intercellular corridor formed by the tight junctional complex (review [159]).

Proximal Tubule

In the proximal tubule, paracellular reabsorption of Ca²⁺ is favored by both the transepithelial electrical potential difference, which is lumen positive under control conditions [160], as well as the transepithelial concentration gradient in which the luminal Ca²⁺ concentration is elevated by isosmotic coupling of water transport to NaCl reabsorption. As a result of the high level of water reabsorption, which approximates 75%, the luminal Ca²⁺ concentration appears to be held above the interstitial Ca²⁺ concentration throughout the length of the proximal tubule.

Thick Ascending Limb

In the TAL, which is an important site of regulated Ca²⁺ transport, NaCl reabsorption occurs independent of water reabsorption and is more tightly coupled to the generation of a lumen positive transepithelial potential difference (Fig. 2.5). The luminal membrane components of the TAL mechanism include Na⁺/K⁺/2Cl⁻ (NKCC2) cotransporters and inwardly rectifying “ROMK” K⁺ channels (review: [96]). The basolateral (blood-facing) components include the Na⁺/K⁺-ATPase, which provides the driving force for transcellular Na⁺ reabsorption, and the CLC-KB Cl⁻ channel, which mediates Cl⁻ efflux from the cell to the interstitium in response to a cell-negative membrane potential difference (review: [96]). Control of Ca²⁺ transport in the TAL arises from regulated expression and transport rates of NKCC2, ROMK, and CLC-KB and is primarily modulated by (1) PTH1Rs, which promote Ca²⁺ reabsorption in response to elevated serum PTH levels and (2) CaSRs, which suppress Ca²⁺ reabsorption (review: [96]).

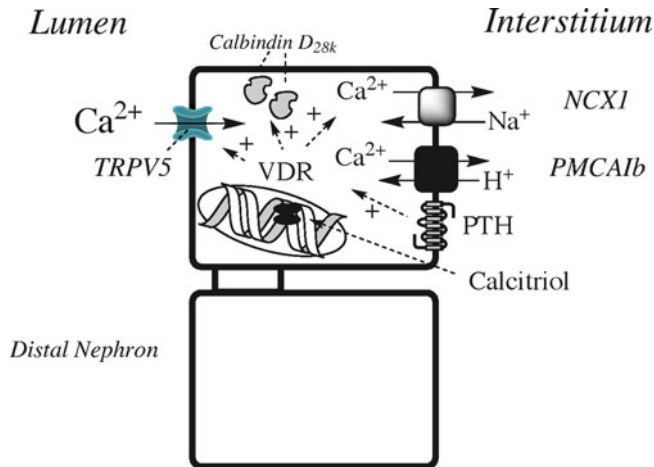


Fig. 2.6 Positive modulation of transcellular calcium reabsorption in the distal nephron by PTH and calcitriol. The mechanism shown in the cell diagram operates in both distal convoluted tubules and connecting tubules (review: [171]). Ca^{2+} -influx across the luminal membrane is mediated by the Ca^{2+} -selective ion channel, TRPV5. Calbindin- D_{28k} and calbindin- D_{9k} (not shown) buffer the cytoplasmic Ca^{2+} concentration and thereby promote continuing Ca^{2+} influx without activating intracellular signaling pathways. Ca^{2+} exits the cell at the basolateral membrane via

PMCA1b and NCX1. PTH and calcitriol both upregulate the expressions of TRPV5, the calbindins, and NCX1 [163, 165]. In addition, PTH activates TRPV5-mediated calcium transport [173]. Although a similar calcium transport mechanism operates in the duodenum, there are key differences between the molecular components (e.g., TRPV6 in the duodenum rather than TRPV5 as here in the distal nephron) and regulation. Thus, although calcitriol promotes calcium transport at both sites, PTH has no direct effect on calcium transport in the duodenum

Distal Convuluted Tubules and Connecting Tubules

Unlike the proximal tubule and TAL, Ca^{2+} reabsorption in the DCTs and connecting tubules, amounting to as much as 15% of the total, is primarily transcellular and dependent on the expression of the TRPV5 Ca^{2+} -selective channel in the luminal membrane, which provides a pathway for Ca^{2+} influx into the cell, together with PMCA1b or NCX1 on the basolateral membrane, which provide a pathway for Ca^{2+} efflux to the interstitium (Fig. 2.6; review: [109]). Ca^{2+} transport is facilitated by expression of the Ca^{2+} chelators, calbindin- D_{28k} (review: [161]), and calbindin- D_{9k} in the cytoplasm of DCT cells. The chelator properties of these proteins appear to prevent the cytoplasmic ionized Ca^{2+} concentration from rising to levels (≥ 200 nM) that might otherwise trigger intracellular signaling events and, by maintaining the steep electrochemical gradient for Ca^{2+} across the apical membrane, facilitate the continuing uptake of Ca^{2+} ions

across the luminal membrane [162]. Interestingly, the cellular uptake of nonprotein Ca^{2+} chelators such as the EGTA-analog, BAPTA-AM mimics the effect of calbindins on calcium transport [162]. The expression of both Ca^{2+} -binding proteins is promoted by calcitriol [163] and, based on studies in cultured kidney cells, the effect of calcitriol on calbindin- D_{9k} appears to be enhanced by PTH [164].

Hormonal Regulation of Renal Ca^{2+} Transport

Parathyroid Hormone

PTH regulates renal calcium excretion by promoting Ca^{2+} reabsorption in the cTAL as well as the DCT and connecting tubule segments of the distal nephron (reviews: [96, 165]). In the cTAL, Ca^{2+} reabsorption is primarily paracellular, accompanied by Mg^{2+} , and antagonized by the CaSR located on the basolateral membrane. In the

DCT and connecting tubule, on the other hand, Ca^{2+} reabsorption is transcellular and PTH promotes the expression and/or functional activity of proteins that support active transepithelial transport. While PTH provides a key drive for stimulated renal Ca^{2+} transport in response to hypocalcemia or in the context of primary hyperparathyroidism, suppression of PTH levels plays a relatively minor role in the counter-regulatory response to hypercalcemia. Instead, CaSR-dependent suppression of TAL Ca^{2+} reabsorption provides a key defense against hypercalcemia [6].

PTH Action in the cTAL

Key determinants of paracellular transport include the concentration gradient between the lumen and interstitium, the transepithelial potential, and the ion permeability of the junctional complex. Although the impact of PTH on NaCl transport in the cTAL is mediated, at least in part, by cAMP-dependent trafficking of NKCC2 to the luminal membrane (review: [96]), PTH is reported to have little or no effect on the transepithelial potential [166] and, thus, on the driving force for transport. Given that *TRPV5* and other key components of the DCT transcellular Ca^{2+} reabsorption mechanism are not expressed in the cTAL (reviews: [96, 155]), it seems reasonable to consider whether PTH stimulation of Ca^{2+} reabsorption in the cTAL arises instead from enhanced Ca^{2+} permeability of the junctional complex, e.g., due to increased expression of claudins.

Claudins are a family of four-transmembrane domain, integral membrane proteins that colocalize with occludins in junctional complexes and are widely expressed in epithelial tissues (review: [111]). They are organized so that both their N- and C termini are intracellular, and form homo- and hetero-oligomers with characteristic isoform-specific expression patterns in renal tubular segments (reviews: [111, 167]). Mutations of claudin-16 or -19 have been linked to a rare human disorder of Ca^{2+} and Mg^{2+} reabsorption in the TAL known as familial hypercalciuric hypomagnesemia with nephrocalcinosis [168] (review:

[167]). Trafficking of claudins to the junctional complexes is regulated, at least in part, by phosphorylation and a PKA site that is required for claudin-16 trafficking has been identified at residue S217 [169].

PTH Action in the DCT and Connecting Tubules

The cloning of *TRPV5* (originally identified as *ECaC-1*) led to its identification as a key component of a transcellular Ca^{2+} transport apparatus in the distal nephron ([108, 170]; reviews: [156, 171]). The cloning of *TRPV6* (originally identified as *CaT1*) represented a similar advance for understanding transcellular Ca^{2+} transport in the duodenum [107]. PTH was previously observed to activate Ca^{2+} uptake across the luminal membrane of distal tubular cells [172], however, the molecular basis for the effect was unknown. It is now clear that PTH promotes both the expression [165] and functional activity [173] of TRPV5. In addition, PTH promotes the expression of calbindin- $\text{D}_{28\text{k}}$ [165], calbindin- $\text{D}_{9\text{k}}$ [164], and NCX1 but not PMCA1b [165]. Thus, PTH is a powerful modulator of the expression of key components required for all rate-limiting steps of transcellular transport in the DCT and connecting tubules (see Fig. 2.6).

Calcitriol

The nature of the effect of calcitriol on renal calcium reabsorption was previously confusing due to its elevation of serum calcium levels and, thus, the filtered load. More recently, the availability of mice that are null either for 1α -hydroxylase or the VDR has permitted a more rigorous assessment of the role of calcitriol in renal calcium handling, leading to the conclusion that it promotes calcium conservation. Thus, VDR null mice exhibit inappropriately high renal Ca^{2+} losses in the context of hypocalcemia, despite the presence of secondary hyperparathyroidism [174]. In addition, 1α -hydroxylase null mice exhibit pronounced decreases in the expressions of key transport proteins of the distal nephron including TRPV5, calbindin $\text{D}_{28\text{k}}$, and the basolateral Na^+ /

Ca²⁺ exchanger, NCX1, which were corrected upon administration of exogenous calcitriol ([163]; see Fig. 2.6).

Calcitonin

Taken together with its well-recognized action in lowering serum Ca²⁺ concentration [175], the apparently paradoxical positive effect of calcitonin on renal calcium reabsorption suggests a primary role in redirecting calcium to bone and retaining it in established hydroxyapatite stores. Consistent with this notion, osteoclast-specific ablation of the calcitonin receptor in mice attenuated their resistance to hypercalcemia [176].

α -Klotho

α -klotho contributes to the control of the renal transport of both calcium and phosphate and is expressed at highest levels in the DCT [177] and at much lower levels in the proximal tubule [178]. It supports renal calcium transport via direct and indirect mechanisms. Thus, it directly stabilizes TRPV5 expression in the plasma membrane via its endogenous β -glucuronidase activity [86, 87]. Indirect roles, dependent in part on the modulation of serum calcitriol levels, are suggested by analyses of the α -klotho null mouse, which in addition to enhanced intestinal calcium absorption exhibits enhanced renal calcium excretion and renal tubular calcium-phosphate precipitation associated with down-regulated expression of calbindin-D_{9k} and NCX1 [179].

Modulation of Renal Ca²⁺ Transport by the CaSR

Elevated extracellular Ca²⁺ concentration promotes renal calcium excretion independent of its action to suppress PTH levels [6]. Consistent with the notion that the kidney senses and responds to changes in the serum ionized Ca²⁺ concentration, the CaSR is widely expressed in the renal tubules. Expression in the proximal tubule provides a

mechanism for modulating the effects of PTH and dietary phosphate intake on phosphate reabsorption (see below; [180, 181]). Expression in the mTAL provides a mechanism for suppressing NaCl reabsorption that takes the form of reduced expression of NKCC2 and ROMK, thereby disrupting uptake of Na⁺ and Cl⁻ ions, and K⁺ recycling across the luminal membrane as well as reduced expression of CLC-KB in the basolateral membrane, thereby disrupting Cl⁻ efflux into the extracellular fluid. Consistent with this notion, activating mutations of the CaSR induce a form of Bartter syndrome (type V) typified by salt wasting and hypokalemia (review: [96]).

Robust expression of the CaSR in the basolateral membrane of the cTAL [182] provides a mechanism for suppressing Ca²⁺ reabsorption and an important defense against hypercalcemia even in the PTH-null mouse [6]. Although its molecular basis is unclear, the CaSR appears to suppress the activities of NKCC2, ROMK, and Na⁺/K⁺-ATPase via the production of arachidonic acid and 20-HETE [183], thereby suppressing the electrical driving force. Alternatively, the CaSR may negatively modulate the insertion of the divalent cation-selective protein, claudin-16 into the junctional complexes thereby reducing the permeability of the paracellular route [184]. CaSR-dependent suppression of PTH-stimulated Ca²⁺ reabsorption in the cTAL arises, in part, from suppression of cAMP levels [185] either by activation of the heterotrimeric G-protein G_i or intracellular Ca²⁺-dependent inhibition of type-6 adenylyl cyclase or intracellular Ca²⁺-dependent activation of type-1 PDE (review: [96]).

Renal Tubular Phosphate Transport

Renal phosphate reabsorption localizes to the proximal tubule and is mediated primarily by the type-II transporters Npt2a and Npt2c, and to a lesser extent, by the type-III transporter, PiT-2 (review: [112]). The significance of Npt2a is underscored by the phenotype of Npt2a null mice, which includes frank renal phosphate wasting, hypophosphatemia, impaired trabecular bone development, elevated serum calcitriol levels,

attendant hypercalcemia and hypercalciuria, as well as secondary hypoparathyroidism [186]. By comparison, Npt2c null mice failed to exhibit significant phosphate wasting, hypophosphatemia or a bone phenotype but instead prior to, but not after, weaning exhibited hypercalcemia and hypercalciuria associated with elevated serum calcitriol levels and reduced renal 24-hydroxylase mRNA expression [187]. Consistent with these findings, Npt2a mediates around 70% of Na/Pi co-transport in murine isolated brush border vesicles (review: [112]) and Npt2c mediates around 30% of renal Na/Pi cotransport in mice fed a low phosphate diet. The supporting role of Npt2c in murine proximal tubular phosphate reabsorption is underscored by the phenotype of double null Npt2a/Npt2c mice, which in comparison to either of the single null mice exhibit aggravated hypophosphatemia, exaggerated metabolic disturbances including an elevated calcitriol level, hypercalcemia, suppressed PTH and FGF23 levels, as well as a severe bone phenotype that includes rickets [188].

Initial analyses of human kindreds with the autosomal recessive disorder, hereditary hypophosphatemic rickets with hypercalciuria, indicated that Npt2c plays the primary role in renal phosphate transport in humans [189, 190]. However, a recent report indicates that an inactivating mutation of Npt2a that interferes with its surface expression can also induce a severe phosphate-wasting phenotype [191].

Regulation of Proximal Tubular Phosphate Transport

Proximal tubular phosphate reabsorption is regulated by both dietary and hormonal factors. Thus, low dietary phosphate promotes and high dietary phosphate suppresses the expression of proximal tubular phosphate transporters including Npt2a, Npt2c, and PiT-2 in the luminal brush border membrane (reviews: [112, 192]). Various hormonal factors modulate proximal tubule phosphate transport including the phosphaturic factors: PTH, FGF-23, secreted frizzled-related protein-4 (sFRP-4), and FGF-7 (reviews: [41, 112]). Calcitriol does not appear to modulate proximal tubule phosphate transport (review: [112]).

Regulation of Proximal Tubular Phosphate Transport by PTH

PTH acutely suppresses the surface expression of Npt2a in the luminal brush-border membranes of proximal tubule cells within minutes (see Fig. 2.7) and has more delayed effects on the surface expression of Npt2c and PiT-2 [24, 193]. PTH acts on type-I PTH receptors (PTH1Rs) expressed on both luminal and basolateral membranes of proximal tubule cells and stimulation of both PI-PLC, arising from the activation of luminal receptors, and adenylyl cyclase, arising from the activation of basolateral receptors, appear to contribute to the response (review: [112]). Signaling mechanisms downstream of PTH1R in the proximal tubule are dependent upon Na⁺/H⁺-exchanger regulatory factors (NHERFs), which interact via tandem PDZ domains with ion transporters including Npt2a as well as various GPCRs, including PTH1Rs (review: [112]). Consistent with a key role of NHERF-1 in proximal tubular phosphate transport, NHERF-1-null mice exhibit defective expression of Npt2a transporters along with phosphate wasting, hypophosphatemia, hypercalciuria, and osteomalacia [194], a phenotype that closely resembles that of Npt2a null mice [186]. NHERF1 stabilizes luminal membrane expression of Npt2a and modulates PTH action by promoting PTH1R-dependent activation of PI-PLC [195]. Recent work suggests that PTH triggers PKC-dependent phosphorylation of NHERF1, disabling its interaction with Npt2a thereby inducing Npt2a internalization. A recent report indicates that NHERFs may also contribute to the regulation of Npt2c and/or PiT-2 [196]. PTH has indirect as well as direct effects on renal phosphate transport, e.g., by promoting the expression of FGF-23 [26].

Regulation of Proximal Tubular Phosphate Transport by FGF-23 and α -Klotho

FGF-23 induces phosphaturia and impairs the renal synthesis of calcitriol [69, 70] via actions on the proximal tubules. The phosphaturic action of FGF-23 is dependent on FGF1c receptors [70] in the presence of the co-receptor, α -klotho

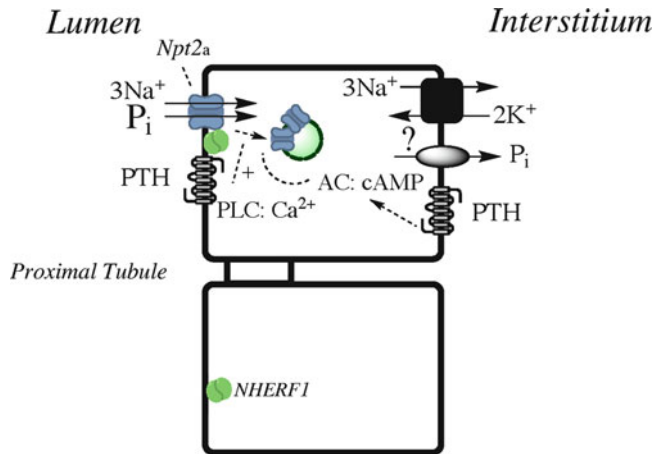


Fig. 2.7 PTH suppresses phosphate reabsorption in the proximal tubule via receptors on both the luminal and basolateral membranes. Transcellular phosphate transport is mediated by Npt2a channels as well as Npt2c and PiT-2 channels (neither of the latter are shown) on the luminal membrane coupled to, as yet, unidentified phosphate channel or transporter on the basolateral membrane. PTH acutely suppresses phosphate transport by disrupting the interaction between Npt2a and

NHERF1 at the plasma membrane. In consequence, Npt2a is rapidly internalized and transcellular transport is markedly inhibited (review: [112]). Npt2c and PiT-2 are inhibited by alternative mechanisms with a much slower time-course. Other modulatory influences on phosphate transport are FGF-23, which like PTH markedly inhibits phosphate transport [70] and luminal calcium-sensing receptors, which dampen the inhibitory effect of PTH [181]

[83, 85] and, like that of PTH, is dependent upon the internalization of Npt2a and Npt2c transporters [70, 72]. However, the actions of FGF-23 on renal phosphate excretion and serum phosphate are delayed in acute studies in humans, i.e., only after 6 h or more [69]. Due to the low level of α -klotho expression in the proximal tubule [177, 178], there has been speculation that the phosphaturic effect of FGF-23 arises indirectly via receptor-dependent expression of paracrine factors from the closely related DCTs ([197]; review: [71]).

Modulation of Proximal Tubular Phosphate Transport by the CaSR

As noted above, the CaSR plays an important role in the inhibitory control of renal calcium reabsorption, which is largely dependent upon its expression in the basolateral membrane of cTAL cells (review: [96]). This mechanism provides a key defense against hypercalcemia [6]. In addition, the CaSR is expressed in the luminal membrane of proximal tubule cells where it reduces the negative impact of PTH on phosphate reabsorption [181]. These results appear to indicate that elevated extracellular Ca^{2+}

levels restrain the phosphaturic response to PTH, thereby limiting the magnitude of phosphate losses and the risk of hypophosphatemia. Interestingly, the introduction of a high phosphate diet or acute exposure to high dose PTH by intraperitoneal injection, both of which induce marked phosphaturia, appear to overcome this restraining mechanism by suppressing proximal tubular expression of the CaSR as well as Npt2a in rats ([180]; review: [92]). The findings suggest that a maximal phosphaturic response requires deactivation of CaSR-dependent inhibition of PTH-dependent signaling as well as decreased surface expression of Npt2a and/or Npt2c.

Novel Hormonal Link: Small Intestinal Phosphate Sensing Drives Renal Phosphate Excretion

Recent work indicates the presence of hitherto unrecognized endocrine interactions between the small intestine and the kidney in the control of phosphate metabolism. In one line of enquiry, instillation of phosphate into the rat small intestinal lumen induced prompt phosphaturia in the absence of an elevation in the serum phosphate

level [198]. The phosphaturic effect was unaffected by parathyroidectomy or renal denervation, and occurred independent of changes in the serum levels of FGF-23 or two other phosphatonins, secreted frizzled-related protein-4 (sFRP4) or FGF-7. Consistent with the notion of phosphate-dependent, small intestinal production of a hormonal regulator of renal phosphate transport, infusion of a duodenal extract induced prompt phosphaturia [198]. In a second line of enquiry, homozygous null *Npt2b* mice, in which small intestinal phosphate absorption was markedly impaired, exhibited only a modest fall in the serum phosphate level in association with upregulated expression of renal proximal tubular *Npt2a* transporters and reduced serum FGF-23 levels [199]. The results indicate that renal phosphate reabsorption can compensate for impaired intestinal phosphate absorption. Finally, overexpression of a stable mutant of FGF-23, R179Q that retains full biological activity, was recently reported to suppress the expression of small intestinal *Npt2b* in wild-type mice but not in mice that were null for either of the proximal tubular transporters *Npt2a* or *-2c*, despite comparable suppression of serum calcitriol levels by FGF-23 (R179Q) in all three models [72]. The findings suggest dynamic coupling of phosphate transport in the kidney and small intestine, via currently unrecognized phosphate-sensing and associated effector mechanisms.

Roles of Bone and Cartilage in Calcium and Phosphate Metabolism

In addition to conferring mechanical rigidity on bone and cartilage, hydroxyapatite acts as the major bodily reservoir of calcium that is released in response to systemic calcium deficiency. It is also a major site of phosphate storage but, as described below, has lesser significance for the maintenance of serum phosphate levels. This section focuses on systemic factors that either control mineralization at the hydroxyapatite surface, and/or promote bone resorption. Clearly bone formation and mineralization are dependent upon the number, activity, and locations of osteoblasts and osteocytes. However, the burgeoning field of the

molecular and cellular biology of bone formation has been excluded from the current discussion, except where it is of direct significance for extracellular fluid calcium and phosphate metabolism. For this reason, the author has omitted discussions of the mechanisms that support the anabolic effect of intermittent PTH including key modulators of osteoblastic bone formation. This extends to negative modulators including serotonin (review: [200]), β -adrenergic agonists (review: [201]), and glucocorticoids (review: [202]) as well as positive modulators including PTHrP (review: [203]), PTH (review: [204]), and IGF-1 (review: [205]) together with their interactions [206]. Thus, the sections that follow focus on the mechanisms that control bone resorption, thereby controlling the release of calcium and phosphate, and mineralization, thereby controlling the transfer of calcium and phosphate to bone.

Significance of the Hydroxyapatite Store in Mineral Metabolism

Although hydroxyapatite is a crystalline phosphate salt of calcium, there is no evidence that it buffers the serum phosphate concentration or corrects hypophosphatemia in the same way that it buffers the extracellular Ca^{2+} concentration or corrects hypocalcemia [207]. Thus, enhanced bone resorption is a key physiological response to hypocalcemia but there is no comparable resorptive response to hypophosphatemia. Instead, hypophosphatemia markedly impairs growth plate maturation and bone mineralization thereby restricting inorganic phosphate transfer from the extracellular fluid to bone (review: [105]). In addition, soft tissue phosphate stores in liver and muscle, contribute to the buffering of serum phosphate levels via mechanisms that are not well understood (review: [208]).

Significance of the Hydroxyapatite Store in the Context of Calcium Deficiency

The significance of the hydroxyapatite store for calcium metabolism is underscored by the development of acute hypocalcemia in subgroups

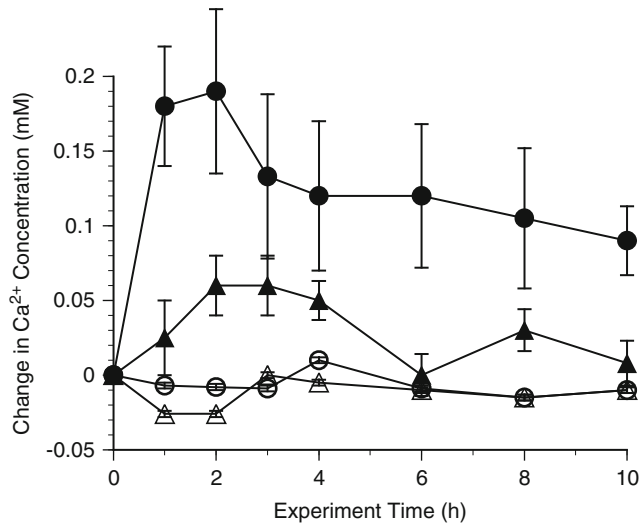


Fig. 2.8 Impaired PTH-induced hypercalcemia in transgenic mice expressing collagenase-resistant type-I collagen. Transgenic *rr* mice that expressed collagenase-resistant type-I collagen exhibited markedly impaired bone resorption and calcium release in response to intraperitoneal injections of PTH. The data

have been redrawn from [211]. *Circles*, wild-type mice; *triangles*, transgenic *rr* mice; *open symbols*, vehicle controls; *closed symbols*, PTH-treated. A greater than 50% reduction in PTH-induced hypercalcemia was observed in *rr* mice. (From [211]. Reprinted with permission)

of patients treated with agents that selectively disable bone resorption including bisphosphonates (review: [209]) or denosumab, a RANK-ligand neutralizing monoclonal antibody [210]. In addition, transgenic mice expressing collagenase-resistant type-I collagen, which disables bone resorption, exhibit a markedly impaired calcemic response to PTH (Fig. 2.8; [211]) indicating that bone resorption is a key element of the PTH-dependent defense against hypocalcemia. Furthermore, persistently elevated PTH in primary hyperparathyroidism promotes bone resorption and elevates the serum Ca²⁺ concentration in the context of renal Ca²⁺ retention. Due to its promotion of renal phosphate wasting, however, PTH tends to suppress the serum phosphate concentration in the context of bone resorption. In support of this outcome, PTH also stimulates the production of the phosphatonin FGF-23 [26, 82].

Significance of the Hydroxyapatite Store in the Context of Phosphate Deficiency

Although the primary, bone-based, hormone response to systemic phosphate deficiency is

impaired bone mineral formation [105, 212], hypophosphatemia also stimulates calcitriol synthesis and calcitriol, in turn, stimulates intestinal phosphate absorption. Thus, hypophosphatemia arising in *Npt2a* null, *Npt2c* null, and double null mice is associated with rickets and osteomalacia together with suppressed serum FGF-23 and PTH levels, an elevated serum calcitriol level and secondary hypercalciuria [188].

Molecular and Cellular Basis of Bone Resorption and its Regulation

Initial activation of the resorbing compartment at the bone surface requires the action of osteoblast lining cell-derived collagenase on type-I collagen (reviews: [213, 214]). Consistent with this conclusion, collagenase-resistant transgenic mice exhibit markedly impaired bone resorption under both basal and PTH-stimulated conditions [211, 215]. Subsequently, exposed extracellular matrix proteins including fibronectin and osteopontin promote β 1-integrin-dependent activation of osteoblasts to express key activators of osteoclast maturation including intercellular adhesion

molecule-1 (ICAM-1) and receptor activator of NF- κ B ligand (RANK-L) [216]. Osteoblast lining cells also express macrophage colony-stimulating factor (M-CSF; [214]). Bone resorption is completed by the actions of mature osteoclasts, which establish membrane-limited resorption pits into which are released HCl and proteases including cathepsin K and, later, neutral matrix metalloproteinases (review: [217]). The action of acid on hydroxyapatite releases calcium and phosphate in ionized form into the extracellular fluid.

Two main bone resorption scenarios need to be considered. The first relates to the maintenance of bone mass and architecture. In this scenario, resorption is a necessary component of remodeling and is targeted to the repair of areas of microdamage or to provide mechanical adaptation to altered strain. The outcomes include revised bone architecture together with enhanced bone quality and strength (reviews: [213, 214]). In one proposed mechanism, apoptotic osteocytes signal to osteoclasts via surface osteoblasts [218], e.g., by the withdrawal of tonic, sclerostin-mediated inhibition of osteoblast expression of the osteoclast activators M-CSF and RANK-ligand.

The second scenario is of particular interest to the present discussion and relates to the role of bone as a calcium store. This store is accessed upon a drop in the serum Ca^{2+} concentration via the release of CaSR-mediated tonic inhibition of PTH secretion leading to elevated serum PTH levels (review: [5]). A similar situation arises in the context of dysregulated PTH secretion, e.g., in primary hyperparathyroidism or FHH (review: [5]). Although hypocalcemia also promotes the synthesis of calcitriol, this effect is generally considered to be of primary significance for intestinal Ca^{2+} absorption rather than bone resorption (review: [33]). Nevertheless, calcitriol may enhance the responsiveness of osteoblasts and/or osteoclasts to persistent elevation of PTH [35].

PTH-Induced Remodeling

Elevated serum PTH levels induce a general activation of remodeling, which is particularly pronounced on the endocortical surface of cortical

bone leading to increased porosity [219] but also affects trabecular bone [220]. Enhanced expression of RANK-L and decreased expression of its soluble receptor osteoprotegerin (OPG) are key elements of the response that include osteoclastogenesis and attendant bone resorption [221].

PTH1Rs, which respond to elevated serum PTH levels as well as locally synthesized PTHrP, are expressed on cells of the osteoblast lineage including marrow stromal cells, osteoblast lining cells, mature osteoblasts, and osteocytes [222]. In addition, PTH1Rs are expressed on T lymphocytes and recent work demonstrates that T-cell-specific deletion of PTH1Rs in mice protects against bone loss via impaired production of the cytokine TNF- α [19]. TNF- α promotes osteoclastogenesis, at least in part, by enhanced stromal cell expression of RANK-L and reduced expression of OPG [19].

Modulation of Bone Remodeling by Calcitriol

Calcitriol has multiple actions on bone mass and mineralization. As noted above, the most important of these actions appear to derive from the promotion of intestinal calcium and phosphate absorption since many of the phenotypic features of 1α -hydroxylase null mice and VDR null mice are corrected by rescue diets rich in calcium, phosphate, and macronutrients (review: [33]). Nevertheless, VDRs are expressed in cells of both the osteoblast and monocyte-macrophage-osteoclast lineages and promote bone formation, at least in part, by enhancing the proliferation of osteoblast progenitors [223], as well as bone resorption, by facilitating the expression of RANK-L [224]. These effects appear to be more pronounced with aging [223] and suggest a role for calcitriol in promoting bone remodeling and turnover (review: [35]). Interestingly, the outcomes of vitamin D receptor function appear to be critically dependent upon the differentiation state of the cells in which it is expressed. Thus, transgenic overexpression of the VDR in mature osteoblasts under the osteocalcin promoter enhanced bone mass and impaired bone

resorption [225], suggesting a role for VDRs in the local downregulation of osteoclasts once mature osteoblasts have been established in the remodeling zone.

Bone Remodeling by Osteocytes

A role for osteocyte PTH1Rs in PTH-induced bone-resorption is suggested by the impact of over-expressing a constitutively active PTH1R under the control of the osteocyte-specific dentin matrix protein-1 (DMP-1) promoter [226]. Although such a mechanism might involve enhanced osteoclastogenesis and bone remodeling at the bone surface, recent work suggests that osteocytes may directly participate in a wider remodeling process that includes the perilacunar matrix and canalicular network dependent upon the upregulation of key bone resorption genes in osteocytes themselves (reviews: [227, 228]). Consistent with this concept, the volume of the perilacunar space appears to expand during lactation [228]. Thus, although the mechanisms are not well-defined, hormonal control of “osteocytic osteolysis” and osteocytic bone formation may also contribute to the control of whole body calcium and phosphate.

Other Modulators of Bone Resorption

Calcitonin

Calcitonin receptors are class B G-protein coupled receptors [229] expressed by mature osteoclasts [230]. They provide a mechanism by which hypercalcemia-induced calcitonin production impairs bone resorption. Consistent with this concept, both global [175] and osteoclast-specific [176] calcitonin receptor null mice exhibit loss of resistance to induced hypercalcemia. Thus, one key defense against hypercalcemia is based on the activation of the CaSR in thyroid C-cells [231], which promotes calcitonin secretion and, in turn, activates calcitonin receptors in osteoclasts to suppress bone resorption.

Estrogens

Postmenopausal bone loss is responsive to therapy with selective estrogen receptor modulators (SERMs) with a tissue selectivity profile that reduces the risk of major estrogen-related side effects including malignant transformation of mammary epithelial cells (review: [232]). Consistent with this behavior, estrogens negatively modulate bone resorption and bone turnover via effects on osteoclast survival and function, and analyses of transgenic mice indicate that both nuclear receptor subclasses, ER- α and ER- β contribute to the effects (review: [233]). However, the identities of the cell types that coordinate the responses, together with their molecular bases, are not well defined. Estrogens upregulate the expressions of Bcl-2, BMP-2, and OPG in osteoblasts, reduce the expression of cathepsin K in osteoclasts, and suppress the levels of pro-osteoclastogenic cytokines (review: [233]).

β -Adrenergic Signaling Promotes Osteoclastogenesis

In addition to its anti-appetite effect, leptin suppresses bone formation via a hypothalamic relay that activates the sympathetic nervous system [234]. In the periphery, β_2 -adrenergic receptors on osteoblasts suppress proliferation and promote the expression of RANK-L and, thus, induce osteoclastogenesis and bone resorption ([235]; review: [201]). The full physiological significance of this sympathetic nervous system mechanism and the nature of its primary sensing modalities are currently unknown.

Bone Formation and Local Regulation of Bone Mineralization

Bone formation, including expansion of the bone matrix and mineralization, is dependent upon the actions of mature, differentiated osteoblasts that align to the bone surface. Osteoblast maturation is under the control of signals arising from apoptotic

osteocytes including withdrawal of sclerostin, a key inhibitor (review: [213]), together with various systemic signals as noted above.

Mature osteoblasts produce the osteoid collagen scaffold, initiate mineralization by seeding calcium-phosphate-containing matrix vesicles, and promote its nucleation via tissue nonspecific alkaline phosphatase (TNAP), which hydrolyses key local inhibitors including pyrophosphate (review: [236]) and polyphosphates [237]. Inactivating mutants of TNAP underlie the human inherited disorder, hypophosphatasia [238] and TNAP-null mice exhibit similar phenotypic features including rickets due to developmental arrest in epiphyses, as well as osteomalacia [239]. Although matrix vesicle-dependent calcium phosphate seeding occurs normally, extension of nascent hydroxyapatite crystals is arrested in the absence of TNAP (review: [240]).

Whether localized to the intracortical or endochondral surfaces of cortical bone, or to the trabeculae of cancellous bone, mineralization requires net transfers of calcium and phosphate ions from the plasma to the surfaces of growing hydroxyapatite crystals. Under steady-state conditions in adults around 8 mmol of calcium and 5 mmol of phosphate are transferred to hydroxyapatite per day and are balanced by similar fluxes of calcium and phosphate arising from bone resorption (Fig. 2.2; [207, 208]).

Control of Mineralization

Critical to the targeting of mineralization is its restriction to discrete sites that support bone integrity with avoidance of joint capsules and joint spaces. Thus, molecular mechanisms for suppressing inappropriate mineralization and for selectively deactivating them in a spatially and temporally appropriate manner are critical to effective growth and/or repair of bone. In addition to TNAP, the pyrophosphate exporter Ank, which is expressed by chondrocytes [241, 242] and osteoblasts [243] and exports pyrophosphate into the extracellular space, is an important contributor to the local inhibitory mechanism. Interestingly, pyrophosphate appears to have multiple inhibitory effects on mineralization that

arise from mineral binding, upregulated expression of osteopontin, and suppressed TNAP activity [244].

Osteoblasts also modulate hydroxyapatite expansion by releasing SIBLINGs that bind to Ca^{2+} ions at the crystal surface [236]. They include DMP-1, bone sialoprotein, osteopontin, and MEPE. While these proteins typically act as inhibitors of hydroxyapatite formation in their full-length forms, phosphorylation and proteolytic processing yields peptides, which (1) promote mineralization, (2) yield more potent inhibitors of mineralization, and/or (3) enter the circulation to modify phosphate metabolism. Dmp1 provides an example of such an inhibitor, which in an intact phosphorylated form binds to hydroxyapatite and suppresses mineralization [245]. However, upon dephosphorylation and proteolytic processing, a 57-kDa C-terminal peptide is generated that promotes mineralization [245, 246].

SIBLING proteins are also modified by limited proteolysis to generate intensely acidic (i.e., negatively charged) peptides. These so-called ASARM peptides negatively modulate hydroxyapatite crystal growth [247] and upon their release into the blood, promote renal phosphate excretion to lower serum phosphate levels by down-regulating the expression of Npt2a (review: [236]). An example of potential clinical importance arises in the context of the cleavage of osteopontin-derived ASARM peptides by the integral membrane endopeptidase, PHEX. Inactivating mutations of PHEX underlie X-linked hypophosphatemic rickets (review: [41]) and failure of PHEX cleavage of phosphorylated osteopontin results in the accumulation of a potent phosphopeptide inhibitor of mineralization [248]. Whether this, or a related, peptide promotes phosphate wasting, another key element of the phenotype, is currently unknown.

Rickets and Osteomalacia: Impact of Low Serum Phosphate

Rickets arises from impaired apoptosis of hypertrophic chondrocytes in the growth plate [249] resulting in a failure of vascularization and

ossification. The serum phosphate concentration is critical to the process and hypophosphatemia induces the persistent survival of hypertrophic chondrocytes suggesting the existence of a phosphate-sensing mechanism coupled to the activation of apoptosis (review: [105]).

Osteomalacia arises from impaired mineralization of osteoblast- or osteocyte-derived osteoid and is also highly sensitive to the serum phosphate concentration [250]. In addition to its inhibitory effect on mineralization arising from the lowering of the calcium-phosphate solubility product, low serum phosphate concentration suppresses the maturation of osteoblasts as revealed by reduced expression of osteocalcin and osteopontin (review: [105]).

Hypophosphatemic Rickets

Several human inherited disorders feature hypophosphatemia and rickets. These include X-linked hypophosphatemic rickets (XLH), ADHR, and various forms of autosomal recessive hypophosphatemic rickets (ARHR). Hypophosphatemia has been causally linked to abnormalities of the growth plate and bone in all of these disorders. In part, it arises from impaired proteolytic processing and/or enhanced expression of FGF-23.

ADHR arises typically from gain-of-function mutations in the FGF-23 gene that disable a critical RXXR proteolytic cleavage site (residues 176–179; [63, 251]). Serum FGF-23 levels are elevated and hypophosphatemia results from renal phosphate wasting and an inadequate intestinal absorption response secondary to suppressed serum calcitriol levels. In addition to persistence of the active, full-length form of FGF-23, impaired processing interferes with the production of a potent C-terminal peptide antagonist of full-length FGF-23 [252].

XLH arises from inactivating mutations in the *PHEX* gene [253–255] and is also associated with elevations of the serum FGF-23 concentration [64]. The *Hyp* mouse, which has an inactivating mutation of the *PHEX* gene, exhibits a comparable phenotype to human XLH including elevated serum FGF-23 levels [256]. These phenotypes were initially ascribed to a failure of FGF-23 processing by defective *PHEX* [64] but *PHEX*

does not cleave FGF-23 and instead appears to negatively modulate its expression [257]. Possibly via the processing of SIBLINGs as described above. Consistent with the notion that enhanced serum FGF-23 levels mediate hypophosphatemia in the *Hyp* mouse, *fgf23/Hyp* double null mice, in which FGF-23 production is ablated, exhibit renal phosphate retention and hyperphosphatemia, i.e., a reversal of the *Hyp* phenotype [258, 259].

ARHR arises from homozygous or compound heterozygous mutations of several genes in humans. These include inactivating mutations of the *Npt2c* or *Npt2a* genes (review: [260]) as noted above, as well as inactivating mutations of the *Dmp1* gene, which exhibit hypophosphatemic rickets and enhanced serum FGF-23 levels ([189]; review: [261]). Consistent with this, *Dmp1* null mice exhibit hypophosphatemic rickets associated with elevated serum FGF-23 levels and renal phosphate wasting [262]. The significance of elevated FGF-23 levels and attendant suppression of the serum phosphate level is underscored by the findings that FGF-23 neutralizing antibodies or a high phosphate diet rescues key features of the phenotype. These include rickets, osteomalacia, and delayed marrow cavity formation, as well as impaired osteoblast to osteocyte differentiation as reported by enhanced expression of *Osterix* (*OSX*) and *Col-1*, and reduced expression of *SOST*, which encodes the key inhibitory regulator of osteoblast differentiation, sclerostin [263]. Taken together, the results suggest that *Dmp1*, like *PHEX*, is a negative regulator of FGF-23 expression. The nature of the interactions between key regulators of osteoblast maturation and mineralization, on the one hand, and enzymes responsible for their proteolytic processing, on the other, remain to be determined. Nevertheless, it seems clear that limited proteolysis of *Dmp1* like that of other SIBLING proteins is critical to its actions in bone [246].

Modulation of Bone Formation and Resorption by the Calcium-Sensing Receptor

Early cellular and transgenic mouse studies aimed at exploring a possible role of the CaSR in bone cell biology led to considerable confusion

(review: [15]). More recent analyses based on tissue-specific deletion of CaSR exon-7, which encodes the receptor's heptahelical signaling domain [264] rather than global deletion of CaSR exon-5, which encodes a C-terminal fragment of the receptor's VFT nutrient-sensing domain [265], has led to a new appreciation of the roles played by the CaSR expressed in the osteoblast, chondrocyte, and monocyte–macrophage–osteoclast lineages with developmental implications for bone and cartilage as well as functional implications for bone remodeling. With respect to bone, homozygous deletion of CaSR exon-7 in osteoblasts under the control of either the *Col-1* or *OSX* promoters induced growth retardation and impaired mineralization associated with defective expression of type-I collagen, alkaline phosphatase, and osteocalcin [264]. Consistent with these findings, the CaSR inhibitor (calci-lytic) NPS 89636 prevented extracellular Ca^{2+} -dependent upregulation of key markers of osteoblast function in cultured fetal rat calvarial cells [266]. The results indicate that the CaSR, in the presence of physiologically relevant extracellular Ca^{2+} concentrations, promotes the differentiation of osteoblast progenitors. Furthermore, examination of neonatal global CaSR/PTH double null mice indicates that the CaSR is required for dietary calcium-dependent stimulation of bone turnover [267]. Thus, the CaSR may mediate key nutritional influences on bone development and bone mass (review: [104]). Surprisingly, expression of a constitutively active CaSR under the osteocalcin promoter induced cancellous bone loss in transgenic mice associated with increased osteoclast number and increased osteoclast surface [268]. The physiological significance of these findings requires further study.

The CaSR is also expressed in osteoclasts and their monocyte precursors. Although its significance in osteoclast biology is less well understood, it promotes monocyte differentiation and chemotaxis and provides a mechanism whereby elevated Ca^{2+} concentrations in the proximity of the bone resorption pit may negatively modulate further bone resorption prior to the recruitment, proliferation, and maturation of osteoblasts in support of bone formation (review: [93]).

Conclusion

When the author agreed to write this chapter, he thought he had undertaken to write an account of the regulation of calcium metabolism alone. That would have simplified life somewhat but would also have been a shame, since this story is really only complete when the metabolisms of both calcium and phosphate are considered together in detail. The story is necessarily complex and involves multiple small intestinal and renal tubular segments, as well as the development and homeostasis of bone and cartilage, together with hormonal and more local molecular signals, some yet undiscovered, that precisely control the functions of these tissues and their interactions. Much of the story points to the roles of micronutrient sensors including the CaSR, which has pluripotent roles in the gut, kidney, bone, and cartilage as well as other sensors, such as those for inorganic phosphate, not yet identified. Ultimately, it provides a picture of an asymmetric economy in which calcium and phosphate are common treated very differently from one another. The positive impact of PTH on serum calcium levels and its neutral or negative impact on serum phosphate levels provides one example. In addition, when their plasma levels are disturbed, calcium and phosphate provoke quite distinct outcomes on bone turnover. The cases of hypocalcemia and hypophosphatemia are particularly striking since the former provokes bone resorption and the latter arrests bone mineralization. There are notable exceptions: the intestinal absorptions of calcium and phosphate are both positively modulated by calcitriol; and calcium and phosphate belong together in the hydroxyapatite crystals in bone. Consideration of all these effects ultimately points to vitamin D and its active metabolites 25-hydroxyvitamin D and calcitriol as central regulators of calcium and phosphate metabolism. The metabolism of calcitriol, in particular, is salutary since it is subject to sensitive and potentially competitive control by all other key players including calcium, phosphate, PTH, and FGF-23. This places the regulation of intestinal calcium and phosphate absorption with its implications

for bone mineralization at the very heart of the metabolic web. Clearly, however, there is much still to learn. What are the molecular identities of the phosphate sensors? How does the proteolytic processing of hydroxyapatite binding proteins control phosphate metabolism? What are the mechanisms that underlie the macronutrient modulation of calcium and, perhaps, phosphate transport? How do osteocytes coordinate the actions of bone remodeling cells and participate themselves in the processes of bone turnover? How does the nutritional modulator, 25-hydroxyvitamin D, interact with key control points in calcium and phosphate metabolism? And how can all of the context-specific variations that perturb calcium and phosphate metabolism independently of one another be explained?

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